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5-HT_{1A} receptor antagonist 8-OH-DPAT attenuates passive avoidance in the elevated T-maze induced by acute stress

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Acute stress exposure induces long-lasting effects on animal behaviour and neuronal plasticity. Previous stressful experiences can modify the animal's responses to new aversive stimuli. Furthermore the reduction of serotoninergic activity provokes anxiolytic effects, counteracting the behavioural consequences induced by stress. This work studies the behavioural long-term consequences of acute stress in rats and the effects of 5-HT_{1A} receptor activation on the behavioural consequences of the acute immobilization (IMMO).

Following acclimation, the rats (Sprague-Dawley, 200–250 g) were randomly divided into four experimental groups ($n = 10$ in each group): (1) control group (with identical pre-experimental treatment without either agonist/antagonist administration or IMMO), (2) group submitted to IMMO (3 h in Plexiglas tubes), (3) group submitted to IMMO with 8-OH-DPAT pre-treatment (0.3 mg kg^{-1}, S.C.) 30 min before acute IMMO and (4) group submitted to IMMO (3 h) after administration of the specific 5-HT_{1A} antagonist WAY-100635 (0.3 mg kg^{-1} S.C., 15 min before 8-OH-DPAT pretreatment. Neuroendocrine effects (corticosterone serum levels) of IMMO with and without 8-OH-DPAT pre-treatment were also studied in different groups following the same protocol. The rats submitted to 3 h IMMO were, 24 h later, assessed for their performance in both conditioned (passive avoidance) and unconditioned (escape behaviour) anxiety tests in the elevated T maze. One-way ANOVA was applied for statistical significance between groups using SPSS for Windows. The experiments were performed following the European Communities Council directive (86/609/EEC) for animal care and experimental procedure and the experiments were approved by the Ethical Community for Animal Research of the University of Malaga.

Our results show that pre-exposure to acute IMMO induces long-term behavioural changes in contrast to control rats. These behavioural alterations include a great increase of anxiogenic responses such as exploratory behaviour and passive avoidance response. These responses were counteracted by 8-OH-DPAT pre-treatment and were reversed by WAY-100635 was administered prior to 8-OH-DPAT. Serum corticosterone levels increased during the first hour of acute stress and after 8-OH-DPAT administration.

Our results clearly support the hypothesis that involvement of acute stress is crucial in the formation of anxiety disorders and aversive memories. Moreover, the 5-HT_{1A} receptor stimulation is able to counteract this long-term effects induced by acute IMMO. In this way, it is suggested that there are molecular interactions between 8-OH-DPAT and glucocorticoid receptors in the brain regions underlying these behaviours.

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All procedures accord with current National and local guidelines.

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Subtypes and splice variants of $GABA_B$ receptors in the NTS of normotensive and spontaneously hypertensive rats

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Inhibitory transmission mediated via the metabotropic $GABA_B$ receptor is believed to be important in control of cardiovascular reflexes at the level of the nucleus of the solitary tract (NTS) and has been implicated in hypertension (Sved & Sved, 1989; Tsukamoto & Sved, 1993; Durgam *et al.* 1999). There are two subtypes of the receptor, $GABA_BR1$ and $GABA_BR2$, with numerous splice variants of the rat R1 subtype having been cloned (1a–1g). In this study, we investigated the expression of known $GABA_B$ receptor variants in the NTS of spontaneously hypertensive (SHR), normotensive Wistar-Kyoto (WKY) and Wistar adult (120–150 g) rats.

Rats were humanely killed by decapitation under halothane anaesthesia (5 % in O_2) and tissue microdissected from the caudal NTS. RNA was reverse transcribed to cDNA. Primers designed to be specific for the $GABA_B$ subunit variants were co-amplified with the ubiquitous internal standard GAPDH in the presence of Taq DNA polymerase, for 28 cycles, determined to be within the exponential phase for all primers. PCR products were separated on 2 % agarose gels and densitometric analysis was made using NIH software Image J v1.2. Results were normalised to GAPDH and for the R1a, R1b and R2 subunits the SHR value was compared to its WKY control. The veracity of PCR products was verified by endonuclease digestion and by sequencing with a ABI Prism 3100 Genetic Analyser and Sea Scape software v1.1.

The data suggested that the R1a, b, c, d splice variants are abundantly expressed in the NTS of all strains of rats, along with R2, with R1e and g weakly expressed and R1f absent. As a positive control, all splice variants were detected in the cortex. The levels of expression of R1b was not significantly different between SHR and WKY, but R1a was significantly increased in SHR ($P < 0.002$, $n = 6$, Student's unpaired t test), as was R2 ($P < 0.03$, $n = 6$, Student's unpaired t test). These data suggest that there is an increase in the expression of $GABA_B$ receptors in the NTS associated with hypertension. This is in agreement with the results of previous pharmacological studies showing enhanced pressor responses to $GABA_B$ receptor activation in the NTS of SHR (Sved & Sved, 1989; Tsukamoto & Sved, 1993), and in acutely hypertensive kidney-wrap rats (Durgam *et al.* 1999). Furthermore, our data suggest a preferential upregulation in the R1a subtype, which we hypothesise may be localised presynaptically on baroreceptor nerve terminals in the NTS.

Durgam VR *et al.* (1999). *Hypertension* **33**, 530–536.

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All procedures accord with current UK legislation.

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Antagonistic galanin–NPY interactions in food intake response: a functional and quantitative receptor autoradiography study

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The aim of this work was to investigate if galanin (GAL) could modulate the feeding responses induced by NPY in satiated rats. We have studied the possible modulation by GAL of the responses elicited by central administration of Y1 and Y2 receptor agonists and also the effect of GAL on their binding characteristics by using receptor autoradiography.

Animals were chronically canulated in the lateral ventricle under sodium pentobarbitone anaesthesia and recovered for 1 week. Food consumption was measured 60 min after intracerebroventricular injections of the Y1 agonist Leu31-Pro34-NPY (2.5 nmol) or the Y2 agonist NPY (13–36) (2.5 nmol) alone or in combination with an effective or a threshold dose of GAL (3.0 nmol and 0.1 nmol respectively). Quantitative receptor autoradiography was performed in brain slices containing the hypothalamus. ^{125}I-labelled Leu31-Pro34-NPY (25 pM) and ^{125}I-labelled PYY (13–36) (25 pM) were used as the Y1 and Y2 agonist, respectively, and the binding was studied in the absence or presence of GAL (1 nM). At the end of the experiments animals were humanely killed.

Feeding responses observed after central administration of the Y1 agonist were counteracted by the presence of a threshold dose of GAL ($P < 0.05$), but no additive effects were found in the presence of an effective dose of GAL. The responses of food intake were not modified by the coadministration of both Y2 agonist and GAL at any of the doses tested. Receptor autoradiography showed that ^{125}I-labelled Leu31-Pro34-NPY binding decreased in the presence of GAL exclusively in the nucleus arcuatus by 30 % ($P < 0.05$); however, GAL was not able to modified the binding of ^{125}I-labelled PYY (13–36) in any of the hypothalamic areas studied.

In conclusion, the functional results demonstrate that GAL antagonizes selectively the feeding responses mediated by NPY Y1 receptor subtypes and the autoradiography data show that GAL decreases exclusively the binding of Y1 agonist. These data evidence that the GAL/NPY antagonism is mediated by the modulation of Y1 receptors probably as a consequence of receptor interactions at the membrane level. This interaction may be of relevance for the understanding the modulation of central control of feeding response by neuropeptides.

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All procedures accord with current National guidelines.